Whole Effluent Toxicity Testing for Management of Toxic Chemicals in Watershed Area

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Abstract. The number of chemicals used in human activities has continuously been increasing, which could deteriorate the habitat of aquatic organisms. In many OECD countries, including the US, France, Germany, and Korea have implemented the Whole Effluent Toxicity (WET) type regulations on industrial/municipal effluents to complement the regulations based on individual toxic chemicals. Our research group has helped the Ministry of the Environment Japan to implement this WET type regulation in Japan and to develop test methods using short-term chronic toxicity tests using three aquatic organisms, fish, daphnia, and algae. Using these methods, we conducted surveys on rivers and streams all over Japan. Approximately 30% and 60% of the waters sampled in Class A rivers all over Japan and in effluent-dominant urban streams, respectively, for at least one organism. General water quality items such as BOD and ammonia were not found to be highly relevant to the detection of the ecotoxicity.

Keywords: Ecotoxicity, Aquatic organisms, Watershed Management.

[1] INTRODUCTION

The number of chemicals registered in Chemical Abstracts Service (CAS) is now nearly 100 million and is still increasing rapidly⁹. Some of these chemicals are used in human activities and eventually released into the environment. However, the number of items in water quality criteria and discharge limit is very limited for most of the countries. For example, only 27 chemicals, mostly toxic heavy metals such as mercury and cadmium, are listed in the items to protect human health and additional 15 items such as biochemical oxygen demand (BOD) are listed to conserve living environment in water quality standards in Japan⁸. In addition, 462 chemicals are listed as class A designated compound to track their production, release, and transfer in the Pollutant Release and Transfer Register (PRTR) system in Japan⁵ while approximately 20,000 chemicals are tested their human health hazards and partly ecotoxicity under Chemical Substance Control Law⁶. Several unregulated (and/or unknown) chemicals including metabolites exist in the aquatic environment and the mixture effects of a million of chemical compounds have become significant concern. Therefore, many countries, most of them are OECD nations, have implemented Whole Effluent Toxicity (WET) type regulation for industrial (and municipal) effluents to control the overall toxicity exerted by the mixture of toxic chemicals in the water environment⁷. The US implemented WET system in 1995 while Germany implemented Wastewater Ordinance in 1976 to protect the habitat of the receiving water bodies. The former uses chronic testing (more sensitive, and In Europe, France has the regulation similar to Germany to charge tax based on acute daphnia toxicity while Sweden, Spain, and North Ireland has the regulation on industrial effluent and the UK is discussing the potential implementation of Direct Toxicity Assessment. In Asia, South Korea and Taiwan implemented the system based on acute toxicity in 2011 and 2013, respectively.

In Japan, Ministry of the Environment started the discussion on the possible implementation of the WET type regulation in 2009 but the discussion seems to be shifted to set only the criteria for self-management by the industries and offices instead of setting discharge limit. National Institute for Environmental Studies cooperated with the Ministry to publicize “Draft testing manual for effluents using bioassay” in 2013⁸. Since the information about the ecotoxicity of ambient water is very limited in the US⁹, our research group has started the research on ecotoxicity of river water using the draft testing method in 2009. We have publicized a couple of papers, one on effluent-dominant urban streams⁹, and the other on over 30 class A rivers all over Japan¹⁰. We have recently been working on the multi-variable analysis of water quality items and ecotoxicity in Yoshino River and Yodo River watershed area. In this paper, we present some of the data obtained in these studies to let the readers to better
understand the current status of the toxic chemicals adversely affecting the habitat of the water environment. This bioassay-based management of toxic chemicals can not only complement the current regulations based on individual chemicals but also substitute some costly measurements of hundreds of potential toxicants.

[2] MATERIALS AND METHODS

2.1. Sample Collections

River water samples were collected in 2011-2012 and 2010-2013 for 30 Class A rivers all over Japan and urban streams in Tokushima, Kyoto, and Saitama, respectively. Additional samplings were conducted in 2013-2015 in Yoshino River watershed area of Tokushima and Yodo watershed area in Kyoto and Osaka to clarify the seasonal variations and the relevance with the water quality items such as BOD and ammonia. All the samples were collected as grab mostly from the center of the flow from the bridge. Otherwise, the samples were collected from riverside. The water samples were stored in refrigerator (4°C) until the start of the toxicity tests.

2.2. Measurement of Water Quality Items

pH, dissolved oxygen (DO), electric conductivity (EC) were measured using portable analyzers (HORIBA D55, HACH HQ30d, and TOADKK CM31P, respectively). Biochemical oxygen demand (BOD), chemical oxygen demand (COD$_{\text{O}}$), ammonia, and total hardness were measured according to standard methods with slight modifications.

2.3. Whole Effluent Toxicity Testing for Riverwater Samples

Toxicity tests were conducted according to “Draft testing manual for effluents using bioassay” publicized by National Institute for Environmental Studies as summarized in Table 1. As can be seen, these methods were designed based on WET test methods of US Environmental Protection Agency with slight modification referring OECD guidelines for testing chemicals. Short-term chronic toxicity tests are proposed instead of acute test because the current trend of chemical management in OECD countries (e.g., REACH in the EU) to use chronic toxicity, which has higher relevance with the maintenance of population of the species.

![Table 1 Overview of the Whole Effluent Toxicity Testing using Three Aquatic Organisms.](image)

<table>
<thead>
<tr>
<th>Name of the Methods</th>
<th>Short-term toxicity test of fish embryo-sac fry stage</th>
<th>Daphnia reproduction test</th>
<th>Algal growth inhibition test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test organisms</td>
<td>zebrafish (Danio rerio)</td>
<td>Ceriodaphnia dubia</td>
<td>Unicellular green alga (Pseudokircheriella subcapitata)</td>
</tr>
<tr>
<td></td>
<td>Duration 9 days</td>
<td>8 days</td>
<td>72 hours</td>
</tr>
<tr>
<td></td>
<td>Dilutions 100% (95% for algae), 50%, 25% and Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endpoint Hatching ratio Mortality</td>
<td>Number of neonates Mortality Growth inhibition ratio</td>
<td></td>
</tr>
</tbody>
</table>

[3] RESULTS AND DISCUSSIONS

While the results of effluent-dominant streams revealed that approximately 60% of the river water samples are toxic to at least one of the three organisms. Daphnia reproduction was significantly inhibited in some urban streams in Tokushima and Saitama, both highly contaminated by untreated greywater. The concentrations of the selected surfactants and pharmaceuticals (and personal care products) were measured and the daphnia toxicity was found to be partly because of the surfactant while some antimicrobials were found to be one group of major contributors.

The results of ecotoxicity tests for water samples in 30 Class A rivers are shown in Figure 1. As can be seen, the frequency of detection was approximately 30% for at least one of the three organisms. The relatively strong toxicity was found for Ishikari Ohashi Bridge of Ishikari River in Hokkaido for all three species. Some other sampling points of detecting strong toxicity include Sasame Bridge of Arakawa River and Takumi Bridge of Ayase River of Tokyo and Tokura Bridge of Yodo watershed area. Characterization of toxicants was conducted for some limited points including these three with repeated ecotoxicity tests. Since the sampling and
testing was conducted only once in most of the sites, and seasonal variation and toxicity identification is necessary for the sites with repeated detection of toxicity.

Since moderate correlation was found for the relationship between water quality items (ammonia and BOD) and daphnia toxicity, we further examined the relationship between the water quality items (pH, DO, BOD, COD, ammonia, and hardness) and algal/daphnia toxicity. The result of principle component analysis is shown in Figure 2. As can be seen, BOD, COD, ammonia, EC, and hardness all are in similar regions with relatively high PC1, which suggest the overall contamination by human activities. Daphnia reproduction and inhibition are in similar regions with higher PC2 and algal toxicity is near the origin. These results suggest the daphnia toxicity is very specific and has weak relationship with the general water quality items such as BOD.

[4] CONCLUSIONS
Our results of short-term chronic toxicity tests for riverwater suggest the reasonable frequency of detection with approximately 30% for class A rivers and 60% for effluent-dominant urban streams. The WET type ecotoxicity testing will provide direct information about the risk of riverwater exerted by toxic chemicals. Since the number of chemicals is increasing, the systems with monitoring both individual toxic chemicals and bio-assay are useful to cover mixture effects and unregulated/unknown compounds. Further investigation is necessary to characterize the toxicants of concern and possibly toxicity identification with chemical analysis is required. This system can be applied to those countries with no proper regulations on chemical management, such as in Southeast Asia. We are currently working on some projects under Asian Core Program in Malaysia to better manage the risk of toxic chemicals in the unit of watershed area.

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